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Life-stage related responses to combined effects of acclimation temperature and humidity on the thermal tolerance of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae)

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ABSTRACT

Adaptive thermal plasticity plays a key role in mitigating the effects of seasonal and diurnal thermal fluctuations among ectotherms at various life-stages. While the role of thermal history in conferring such plasticity is widely documented, its interaction with relative humidity (RH), another important driver of ectotherm survival and activity, is relatively underexplored. Yet the potential responses to these combinational stressors across ontogeny remain largely neglected. Against this background, we used a full-factorial design to test the combined acclimation effects of RH (45%, 65% and 85%) and temperature (23, 28 and 33 °C) on various indices of thermal sensitivity of laboratory reared spotted stemborer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) following acclimation beginning at larval, pupal and adult life-stages. Traits measured included critical thermal limits (CTLs), supercooling points (SCPs), chill coma recovery time (CCRT) and heat knockdown time (HKDT). Larval acclimation at 23 °C; 85% RH recorded the lowest critical thermal minima (CT_{min}) whereas adult acclimation at 28 °C; 45% RH recorded the highest critical thermal maxima (CT_{max}). There were no significant differences ($P > 0.05$) in SCPs across all temperature × RH acclimations. Larval and pupal acclimations at 23 °C; 85% RH and adult acclimation at 23 °C; 45% RH significantly improved CCRT. Similarly, commencing acclimation at larval, pupal and adult stages at 28 °C; 85% RH improved HKDT whereas larval and pupal acclimations at 33 °C; 45% RH impaired it. Our results indicate that combinational interactions of temperature and RH have significant thermal fitness costs and benefits and are dependent on the life-stage acclimation timing. Results also imply that both the vulnerability and adaptive potential of *C. partellus* populations under rapid climate variability varies with ontogeny. This therefore calls for the consideration of the role of ontogeny and multi-factors in better understanding the impact of environmental stress on ectotherms.

1. Introduction

Environmental perturbations associated with the rapidly changing climate pose serious challenges to ectotherm fitness (Parmesan, 2006). However, many species respond to environmental variability via a suite of mechanisms that enable population persistence under unfavourable environments e.g. through behavioural compensation, or using acclimation, a form of phenotypic plasticity (see Hoffmann and Parsons, 1991). Phenotypic plasticity is the ability of a single genotype to portray a variety of phenotypes in response to different environmental stimuli (Garland and Kelly, 2006; Gibbin et al., 2017) and can involve adaptive adjustments at biochemical, physiological, behavioural and morphological level (Yampolsky et al., 2014; Gerken et al., 2015). For example, under acute thermal variability, ectotherms employ rapidly inducible and reversible physiological responses (e.g. Nyamukondiwa et al., 2013) allowing them to track diurnal thermal fluctuations and to preserve key life-history traits such as reproduction. These are referred to as rapid cold hardening or rapid heat hardening in the case of low or high temperature resistance, respectively (Chidawanyika et al., 2017; Mutamiswa et al., 2018a). Over relatively longer time scales, this adaptive form of phenotypic plasticity can also be employed in a process called acclimation (Lagerspetz and Vainio, 2006) or developmental acclimation where, in the latter, plasticity is acquired during ontogenesis (Chidawanyika and Terblanche, 2011).

Ontogeny has been reported as a major factor influencing the magnitude of phenotypic plasticity in response to abiotic stress (Marais and Chown, 2008; Marais et al., 2009; Arias et al., 2011; Lockwood et al., 2018). Indeed, the duration of acclimation exposure and

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ontogenetic acclimation timing determine the direction of induced plasticity and any associated costs and/or benefits (Bowler and Terblanche, 2008). For insects, this is of particular importance due to the complexity of their life cycles where different life-stages can experience variable microenvironments during development and have differential abilities for behavioural compensation to abiotic stress. Generally, the less or non-mobile life-stages are considered to have evolved inherent high phenotypic plasticity to abiotic stress, to compensate for limited ability of behavioural responses. Indeed, a number of studies have supported this assumption among various taxa (Vernon and Vannier, 1996; Klok and Chown, 2001; Jensen et al., 2007 but see Marais et al., 2009).

Given the potential that plastic evolutionary adaptations have in mitigating the impacts of global climate change among ectotherms, it is not surprising that this has been a subject of interest among physiologists in recent decades (Hemmati et al., 2014; Nguyen et al., 2014; Cavieres et al., 2016). Nevertheless, one aspect currently underexplored is how humidity may influence thermal plasticity levels during ontogeny despite the likelihood of simultaneous fluctuations of these factors under global climate change. Certainly, insect survival and geographic distribution is partially dependent on their ability to withstand variations in body water content elicited by fluctuations in temperature and relative humidity (RH) (Norhisham et al., 2013). This is due to their combined effects on important biological factors such as metabolic rate and development (Hoffman et al., 2012; Nguyen et al., 2014). Because of their small body size, insects are highly susceptible to high water loss in hot and dry conditions associated with low latitude areas (Gibbs et al., 2003; Kellermann et al., 2018). For example, low humidity and high temperatures influence the fitness and survival of tsetse flies with some increasing their range to seek niches with favourable microclimates (Terblanche et al., 2008). In addition, the interaction between high temperature and low RH has been reported to elicit further stressful conditions for *Drosophila melanogaster* compared to high temperature and high RH interaction (Bubliy et al., 2012). This underscores the notion that the interaction of abiotic factors may differentially influence insect thermal sensitivity, fitness and survival.

High humidity may also enhance reproductive capacity in insects as there is a correlation between body water and body fat content, traits that are deemed key for reproductive success (Norhisham et al., 2013). Consequently, insects have to maintain their body water content within certain thresholds through adjustments of e.g. cuticular permeability and hormone secretion (Raghu et al., 2004). With global climates changing, many insects face increasingly novel and sometimes divergent abiotic constraints (May et al., 2018), e.g. increased temperature coupled with variable and stressful humidities. As such, the interactive effects of these factors are likely to play an important role among insects (Vazquez et al., 2015). For example, Southern Africa is projected to experience 10% reduction in precipitation and increased drought frequency while east Africa is expected to experience high precipitation (Lobell et al., 2008; Stathers et al., 2013) and ectotherms in this region will undoubtedly require context-dependent compensatory mechanisms under such climate variability. Insects facing temperature and RH variations may cope through physiological and behavioural mechanisms to improve fitness and survival (Lachenicht et al., 2010). For instance, most insects can improve their metabolic water sources, accumulate high body water content, reduce water loss rates or the regulation of diuretic hormones to survive dehydration stress (Gibbs et al., 2003). Nevertheless, how these water balance adjustments influence thermal acclimation, a key mechanism driving insects' functional niche (e.g. Angilletta, 2009) remains poorly explored.

Chilo partellus (Swinhoe) (Lepidoptera: Crambidae) is a robust invasive economic insect pest of cereal crops responsible for high yield losses (Khadioli et al., 2014). Our previous studies suggest that it may be a useful model organism for exploring how insects cope with integrated stress factors (e.g. Gotcha et al., 2018). Temperature, rainfall and RH have been reported as the main contributing factors towards *C. partellus* biogeography (Sithole, 1989; Khadioli et al., 2014). However, it remains unclear how the interaction of these environmental factors shape critical *C. partellus* fitness traits e.g. temperature tolerance (Angilletta, 2009), population abundance and biogeography under global change (Mutamiswa et al., 2017a). To date, most bioclimatic models on this pest and related taxa mainly focus on single variables e.g. temperature (Mwalusepo et al., 2015) in predicting phenology and biogeography. While some previous studies focused on modeling temperature effects on insect development and other life history parameters (e.g. longevity, oviposition and fecundity) (Khadioli et al., 2014a, 2014b), a few have considered combined temperature and RH effects on the same parameters (Getu, 2007; Tamiru et al., 2012). Moreover, to the best of our knowledge, none have focused on the role of ontogeny on thermal tolerance. The current study therefore unravels how insect thermal fitness is shaped by exposure to divergent climatic constraints and may help explain how invasive species e.g. *C. partellus* cope with novel and continuously changing abiotic environments. Specifically, we investigate the combined effects of RH and temperature during acclimation (of different developmental stages, *vis* larval, pupal and adult) on the thermal tolerance of adult spotted stemborer, *C. partellus* measured as critical thermal limits (CTLs), supercooling points (SCPs), chill coma recovery time (CCRT) and heat knockdown time (HKDT). Occurrence of these simultaneous heterogeneous stressors is increasing under rapidly changing climates (Gotcha et al., 2018) and often has fitness consequences (Renault et al., 2018). Physiological assays measured here are often used as a proxy for determining species fitness responses to changing biotic environments (e.g. Dixon et al., 2009; Kelley, 2014; Renault et al., 2018).

2. Materials and methods

2.1. Insect culture

Chilo partellus initial colonies were obtained as pupae from International Centre for Insect Physiology and Ecology (ICIPE), Kenya. This stemborer species had been in the laboratory for more than 20 generations with regular augmentation with wild populations for gene infusion. Pupae were reared in open Petri dishes in Bugdorm rearing cages (240 mm^3; Bugdorm-BD43030F, Megaview Science Co., Ltd, Taiwan) under 28 ± 1 °C, 65 ± 10% RH and 12 L: 12D photocycle in climate chambers (HPP 260, Memmert GmbH + Co. KG, Germany) until adult eclosion. Following eclosion, adults were provided with water-soaked cotton wool in a 60 ml plastic vial. Wax paper folded into several pleats was placed in the cages as an oviposition substrate for gravid females. To maintain uniformity among test insects, eggs were harvested after every 12 h, and transferred to artificial diet (Ochieng et al., 1985; Tefera et al., 2010) where they were allowed to hatch into larvae until pupation. Pupae were harvested every 24 h and also maintained in open Petri dishes in rearing cages for subsequent generations. Once every month during summer, wild-caught moths were added to the colony to maintain high levels of heterozygosity (e.g. Nyamukondiwa and Terblanche, 2009).

2.2. Acclimation treatments for different developmental stages

Long term larval, pupal and adult acclimation effects were assayed using established protocols modified from Nyamukondiwa and Terblanche (2010). Experiments were arranged in a 3 × 3 factorial design (3 of each temperature and RH acclimation treatments). Acclimations were done at 23, 28 and 33 °C at 45, 65 or 85% RH under 12:12 (L: D) photoperiod to give a total of nine treatments (see Fig. 1).

For all the three life-stages (larvae, pupae and adults), the insects were first randomly partitioned into nine groups and transferred from benign/rearing conditions (28 °C; 65% RH) and held in Memmert climate chambers preset under the temperature-humidity conditions illustrated in Fig. 1. During acclimation, larvae and pupal treatments

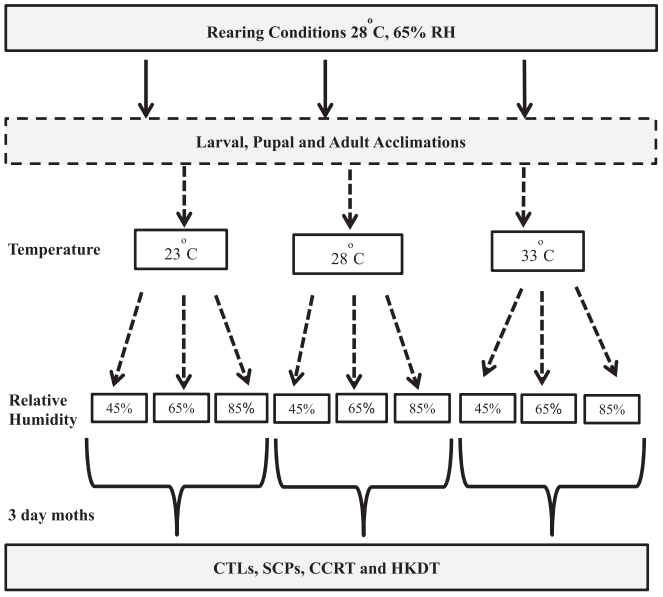

Fig. 1. Schematic diagram for acclimation experimental protocols. CTLs represent Critical thermal limits; SCP- Supercooling point; HKDT- Heat knockdown time and CCRT- Chill coma recovery time. For full description see Section 2.2.

were staggered based on preliminary trials to ensure that adult eclosion was synchronized. Thereafter, all adults were held for a further three days in the respective treatments before running experiments. For adult acclimation treatments, newly eclosed adults from the standard rearing conditions only had acclimation in the first three days post-eclosion before the onset of experiments. This acclimation timing is sufficient to elicit acclimation responses (see Weldon et al., 2011).

In all acclimation treatments, larvae were provided with standard artificial diets and adults moths were provided with wads of cotton moistened with distilled water for drinking ad libitum.

2.2.1. Critical thermal limits (CTLs)

Critical thermal limits (CT_{min} and CT_{max}) were measured following standardized protocols (see Nyamukondiwa and Terblanche, 2010; Mutamiswa et al., 2017b). A series of insulated double-jacketed chambers ('organ pipes') was connected to a programmable water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany) filled with 1:1 water: propylene glycol to allow for subzero temperatures at the same time regulating the flow of liquid around the chambers. Ten adult moths were individually placed randomly into the organ pipes and subjected to constant heating or cooling rate. In the 'chambers', insects were allowed to first equilibrate at 28 °C (equivalent to the benign rearing temperature) for 10 min before ramping temperature up (CT_{max}) or down (CT_{min}) at a rate of 0.25 °C min^{-1}. This was repeated twice with different moths to yield sample sizes of $n = 20$ per treatment. The body temperature of each individual insect was assumed to be in equilibrium with the chamber temperature as in like insect taxa (Terblanche et al., 2007a). A thermocouple (type K 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into a control chamber to measure chamber temperature. CTLs were defined as the temperature at which each individual insect lost coordinated muscle function which was regarded as a lack of response to mild prodding (e.g. Nyamukondiwa and Terblanche, 2010).

2.2.2. Supercooling points (SCPs)

SCPs for adult moths were assayed as outlined by Nyamukondiwa et al. (2013). A total of sixteen moths were individually placed in 0.65 ml microcentrifuge tubes where each insect was placed in contact with the tip of a type-T copper-constantan thermocouple (762–1121, Cambridge, UK). The thermocouples were inserted through the lid of each tube with both insect and thermocouple secured in place by a cotton wool. The thermocouples were connected to one of two 8-channel Picotech TC-08 (Pico Technology, Cambridge, UK) thermocouple interfaces and temperatures were recorded at 1 s intervals using PicoLog software for windows (Pico Technology, Cambridge, UK). In all cases, experiments commenced by holding insects at a set point temperature of 15 °C for 10 mins (to allow equilibration of insect body temperatures) before ramping down at 0.5 °C min^{-1} until SCPs were recorded. In the current study, SCP for each individual was defined as the lowest temperature recorded prior to a spike in temperature associated with the latent heat of crystallization (see Nyamukondiwa et al., 2013).

2.2.3. Chill coma recovery time (CCRT)

CCRT experiments were assayed following larval, pupal and adult acclimations as outlined by Weldon et al. (2011). Ten moths were individually placed in 0.65 ml microcentrifuge tubes and then loaded into a large zip-lock bag that was submerged into a water bath (Systronix, Scientific, South Africa) filled with 1:1 water: propylene glycol set at 0 °C for 1 h. This temperature × time interaction treatment is generally suffice to elicit chill-coma in other insect taxa (see Weldon et al., 2011; Sinclair et al., 2015). Following 1 h at 0 °C, the tubes were immediately transferred from the water bath to the climate chamber set at 28 °C, 65% RH for recovery. This was repeated three times with different moths to yield sample sizes of $n = 30$ (30 replications) per treatment. A video recording camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) linked to a computer was connected to the climate chamber. All observations and data were recorded from this climate chamber video recording system. CCRT was defined as the time (in minutes) required for the moths to regain consciousness, e.g. ability to stand upright on its legs (Milton and Partridge, 2008).

2.2.4. Heat knockdown time (HKDT)

As in CCRT, HKDT experiments were also assayed as outlined by Weldon et al. (2011). Ten replicate organisms were individually placed in 0.65 ml microcentrifuge tubes and placed in a climate chamber connected to a Covert Network Camera that was linked to a computer. The moths were then exposed to a test temperature of 49.0 ± 0.3 °C and 65% RH in the climate chamber. This knockdown temperature (49.0 °C) was selected based on preliminary investigations of upper critical temperatures to activity which was ~47.88 ± 0.76 °C for adults (see also Mutamiswa et al., 2017b). This was repeated three times with different moths to yield sample sizes of $n = 30$ per treatment. All observations were recorded from the climate chamber video recording. HKDT was defined as the time (in minutes) at which activity was lost by the organisms following exposure to heat knockdown temperature (49.0 °C) in a climate chamber (Weldon et al., 2011).

2.3. Statistical analyses

Before analysing CTLs, SCPs, CCRT and HKDT results, data were checked for normality and equality of variances using the Shapiro-Wilk and Hartley-Bartlett tests respectively, and in all cases met linear model assumptions of constant variance and normal errors. As such, results were assessed using full factorial analysis of variance (ANOVA) with CT_{min}, CT_{max}, SCP, CCRT and HKDT being the dependent variables whereas developmental stage (larva, pupa and adult), temperature and RH acclimation were the categorical factors. This was done in STATISTICA, version 13.0 (Statsoft Inc., Tulsa, Oklahoma). Tukey-Kramer's *post-hoc* tests were used to separate statistically heterogeneous groups. Overlap in 95% confidence limits was used to identify statistically homogeneous groups.

Table 1
Summary results from a full factorial ANOVA showing the effects of acclimation stage (larval, pupal and adult), temperature (23, 28 and 33 °C) and relative humidity (45, 65 and 85%) and their interaction effects on critical thermal minima (CT_{min}) and maxima (CT_{max}). SS = sum of squares, DF = degrees of freedom, MS = means of squares.

Trait	Effect	SS	DF	MS	F	P
CT_{min}	Intercept	6217.27	1	6217.27	39,640.53	< 0.0001
	Acclimation stage	9.85	2	4.93	31.41	< 0.001
	Temperature	266.67	2	133.33	850.12	< 0.0001
	Relative Humidity	147.57	2	73.82	470.43	< 0.0001
	Acclimation * Temperature	21.34	4	5.34	34.02	< 0.0001
	Acclimation * Relative humidity	17.98	4	4.5	28.67	< 0.0001
	Temperature*Relative humidity	102.48	4	25.62	163.36	< 0.0001
	Acclimation * Temperature * Relative humidity	18.09	8	2.26	14.42	< 0.0001
	Error	80.46	513	0.16		
CT_{max}	Intercept	1,255,205	1	1,255,205	3,344,120	< 0.0001
	Acclimation stage	11	2	6	15	< 0.001
	Temperature	7	2	4	9	< 0.0001
	Relative Humidity	44	2	22	58	< 0.0001
	Acclimation * Temperature	27	4	7	18	< 0.001
	Acclimation * Relative humidity	8	4	2	5	< 0.001
	Temperature*Relative humidity	23	4	6	16	< 0.001
	Acclimation * Temperature * Relative humidity	15	8	2	5	< 0.001
	Error	193	513	0		

3. Results

3.1. Critical thermal limits

Critical thermal minima (CT_{min}) and maxima (CT_{max}) significantly varied across acclimation type (temperature and RH) and acclimation stage (Table 1; Fig. 2A and B). In addition, acclimation stage, temperature, RH, acclimation stage × temperature, acclimation stage × RH, temperature × RH and acclimation stage × temperature × RH interactions were significant for both CT_{min} and CT_{max} ($P^{\leq}0.001$) (Table 1). At control treatments (28 °C; 65% RH), there were no significant life-stage related responses to acclimation for both CT_{min} and CT_{max} (Fig. 2A; B). Compared to control, there was a significant life-stage difference in CT_{min} across all acclimations at 23 °C; 65% RH, with larval acclimated moths being more cold tolerant relative to pupal and adult acclimated ones (Fig. 2A). Larval acclimation at 23 °C; 85% RH resulted in lowest (most enhanced) CT_{min} recorded relative to pupal and adult acclimations (Fig. 2A). Similarly, significant differences were recorded in CT_{max} between controls and treatments at 33 °C; 85% across all acclimations (Fig. 2). In addition, significant differences in CT_{max} were recorded between larval and adult acclimated moths at 23 and 28 °C; 45% RH (Fig. 2B). Nevertheless, there was no significant difference in CT_{max} ($P > 0.001$) across all acclimations at 23, 28 and 33 °C; 85% RH (Fig. 2B). At 28 °C; 45% RH, adult acclimation resulted in all test organisms recording the highest CT_{max} followed by pupal and larval acclimations (Fig. 2B).

3.2. Supercooling points

There was a significant temperature, acclimation stage × temperature and acclimation stage × temperature × RH interactions effect on SCPs ($P < 0.001$) (Table 2). However, there was no significant acclimation stage, RH, acclimation stage × RH and temperature × RH interactions ($P > 0.05$) (Table 2). At control treatments, there were no significant life-stage related responses to acclimation (Fig. 3). However, significant differences were recorded between controls and treatments at 33 °C; 45% RH and 33 °C; 65% RH following pupal acclimation (Fig. 3). Pupal acclimated moths had significantly more negative SCPs than larval acclimated ones at 33 °C; 65% RH (Fig. 3). However, no significant differences were recorded across all acclimation stages at 23 and 28 °C and their RHs (45%, 65% and 85%) (Fig. 3).

3.3. Chill coma recovery time

Chill coma recovery time significantly varied across acclimation type and acclimation stage (Table 3; Fig. 4). There was a significant acclimation stage, temperature, RH, acclimation stage × temperature, acclimation stage × RH, temperature × RH and acclimation stage × temperature × RH interactions on CCRT ($P < 0.001$) (Table 3). At control treatments, there were no significant life-stage related responses to acclimation for CCRT (Fig. 4). However, there was a significant difference in CCRT for controls and all treatments at 33 °C; 45% and 33 °C; 65% RH (Fig. 4). Larval acclimated moths had shorter recovery times than the adult acclimated ones ($P < 0.001$), while pupal acclimated moths also showed a shorter (more enhanced) CCRT relative to adult acclimations ($P < 0.001$) for 28 °C; 85% RH treatment (see Fig. 4). Similarly, at 33 °C; 45% RH, the larval and adult acclimations showed no significant difference ($P > 0.05$). Nevertheless, these two had a more enhanced (shorter) CCRT compared to pupal acclimations (Fig. 4). However, there was no significant difference in CCRT ($P > 0.05$) across all acclimations at 28 °C; 45% and 65% RH as well as 33 °C; 65% RH (Fig. 4).

3.4. Heat knockdown time

As in CCRT, HKDT significantly varied across acclimation type and acclimation stage (Table 4; Fig. 5). Acclimation stage, temperature, RH, acclimation stage × temperature, acclimation stage × RH, temperature × RH and acclimation stage × temperature × RH interactions significantly influenced HKDT ($P < 0.001$) (Table 4). At control treatments, there were significant differences between larval or pupal acclimation when compared to adult acclimation for HKDT (Fig. 5). The former (larval and pupal acclimation) had no significant differences in HKDT. At 28 °C; 85% RH, adult acclimation significantly improved heat tolerance (HKDT) ($P < 0.001$) relative to both larval and pupal acclimations at 28 °C; 65% RH. Similarly, at 33 °C; 85% RH, adult acclimated moths had longer HKDT (higher heat tolerance), compared to developmentally preconditioned moths (larval and pupal acclimation) (Fig. 5). Moreover, larval acclimated moths at 23 °C; 85% RH had significantly higher heat tolerance (longer HKDT) compared to pupal and adult acclimations at 23 °C; 45% RH (Fig. 5). Furthermore, there was no significant difference in HKDT for moths that were acclimated at 28 °C; 45% RH and 28 °C; 85% RH ($P > 0.05$) (Fig. 5).

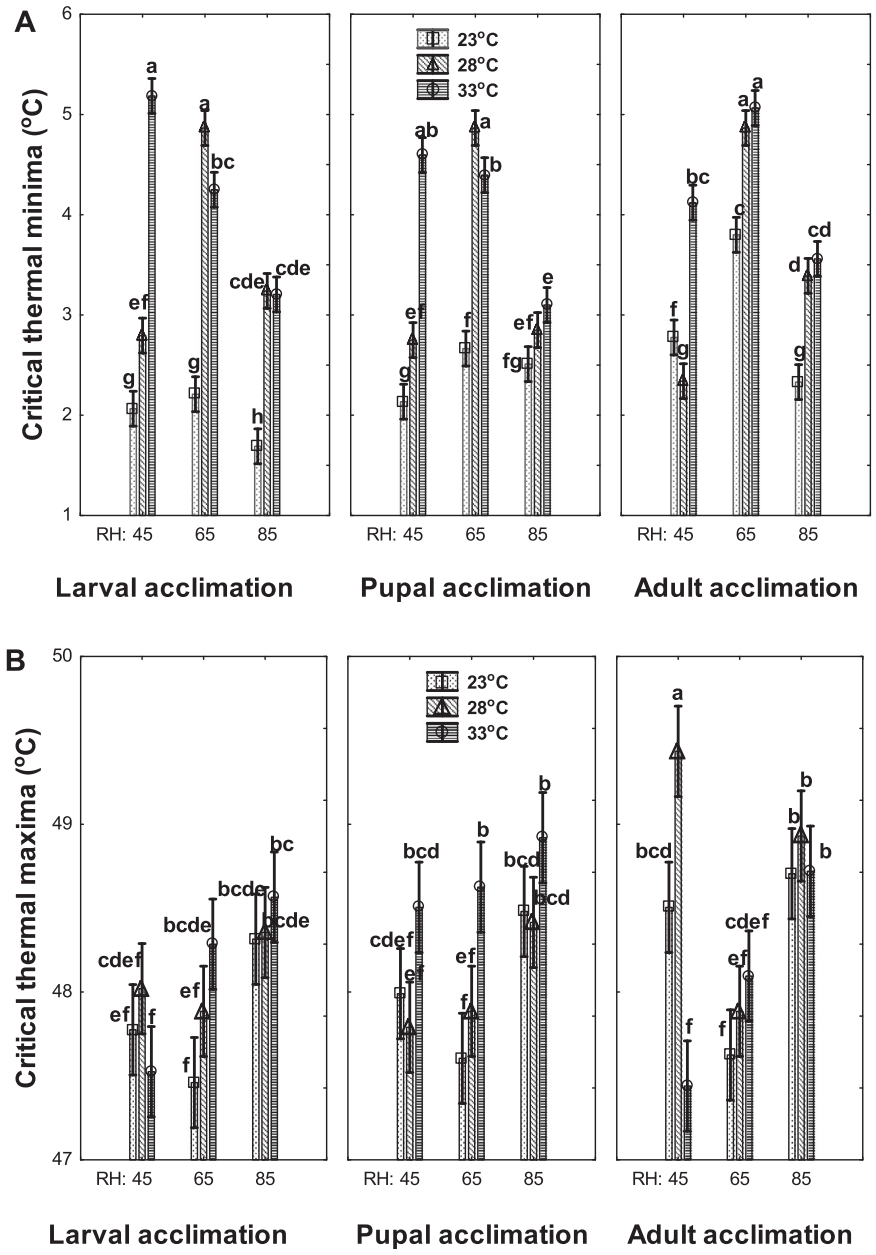

Fig. 2. Summary results of the effects of temperature (23, 28 and 33 °C), relative humidity (45%, 65% and 85%) and acclimation stage (larval, pupal and adult) on adult *C. partellus* (A) Critical thermal minima and (B) Critical thermal maxima. Vertical bars represent 95% CLs (N = 20 per group). Means with the same letter are not statistically different.

Table 2
Summary results from a full factorial ANOVA showing the effects of acclimation stage (larval, pupal and adult), temperature (23, 28 and 33 °C) and relative humidity (45%, 65% and 85%) and their interaction effects on supercooling points (SCP). SS = sum of squares, DF = degrees of freedom, MS = means of squares.

Trait	Effect	SS	DF	MS	F	P
SCP	Intercept	111,525.2	1	111,525.2	28,796.80	< 0.0001
	Acclimation stage	4.8	2	2.4	0.62	0.54
	Temperature	53.0	2	26.5	6.84	< 0.001
	Relative Humidity	35.5	2	17.7	4.58	0.011
	Acclimation * Temperature	73.3	4	18.3	4.73	< 0.001
	Acclimation * Relative humidity	61.2	4	15.3	3.95	0.0037
	Temperature*Relative humidity	67.3	4	16.8	4.35	0.0019
	Acclimation * Temperature * Relative humidity	110.9	8	13.9	3.58	< 0.001
	Error	1568.5	405	3.9		

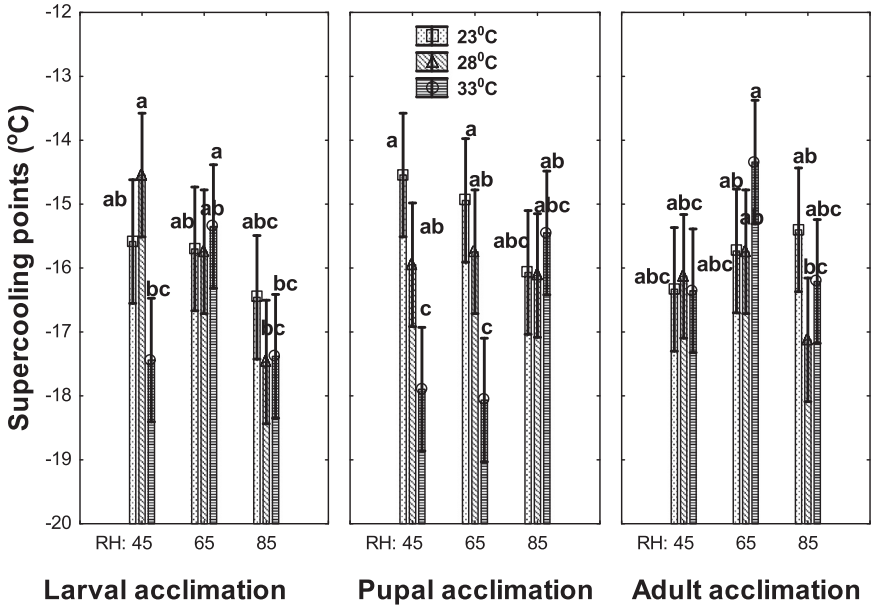

Fig. 3. Summary results of the effects of temperature (23, 28 and 33 °C), relative humidity (45%, 65% and 85%) and acclimation stage (larval, pupal and adult) on adult *C. partellus* supercooling points. Vertical bars represent 95% CLs (N = 16 per group). Means with the same letter are not statistically different.

Table 3
Summary results from a full factorial ANOVA showing the effects of acclimation stage (larval, pupal and adult), temperature (23, 28 and 33 °C) and relative humidity (45%, 65% and 85%) and their interaction effects on chill coma recovery time (CCRT). SS = sum of squares, DF = degrees of freedom, MS = means of squares.

Trait	Effect	SS	DF	MS	F	P
CCRT	Intercept	3686.78	1	3686.78	15,123.81	< 0.0001
	Acclimation stage	3.5	2	1.75	7.17	< 0.001
	Temperature	107.41	2	53.71	220.31	< 0.0001
	Relative Humidity	5.84	2	2.92	11.98	< 0.001
	Acclimation * Temperature	15.94	4	3.98	16.34	< 0.001
	Acclimation * Relative humidity	10.46	4	2.61	10.72	< 0.001
	Temperature*Relative humidity	8.63	4	2.16	8.85	< 0.001
	Acclimation * Temperature * Relative humidity	33.98	8	4.25	17.42	< 0.0001
	Error	190.88	783	0.24		

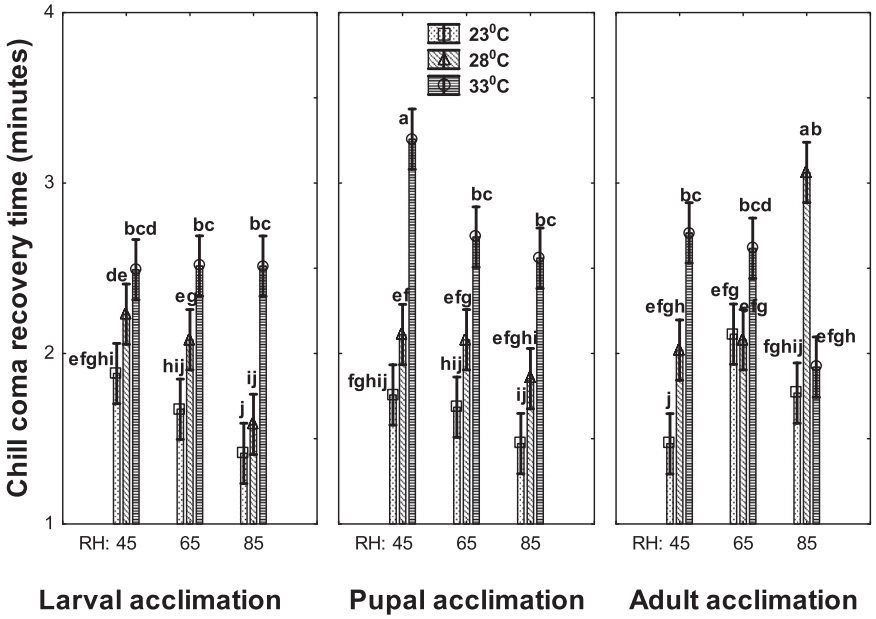

Fig. 4. Summary results of the effects of temperature (23, 28 and 33 °C), relative humidity (45%, 65% and 85%) and acclimation stage (larval, pupal and adult) on adult *C. partellus* chill coma recovery time. Vertical bars represent 95% CLs (N = 30 per group). Means with the same letter are not statistically different.

Table 4
Summary results from a full factorial ANOVA showing the effects of acclimation stage (larval, pupal and adult), temperature (23, 28 and 33 °C) and relative humidity (45%, 65% and 85%) and their interaction effects on heat knockdown time (HKDT). SS = sum of squares, DF = degrees of freedom, MS = means of squares.

Trait	Effect	SS	DF	MS	F	P
HKDT	Intercept	114,669.1	1	114,669.1	49,063.9	< 0.0001
	Acclimation stage	257.9	2	128.9	55.17	< 0.0001
	Temperature	116.8	2	58.4	25	< 0.001
	Relative Humidity	1431.3	2	715.7	306.21	< 0.0001
	Acclimation * Temperature	427.6	4	106.9	45.74	< 0.0001
	Acclimation * Relative humidity	88.8	4	22.2	9.5	< 0.001
	Temperature*Relative humidity	323.9	4	81	34.65	< 0.0001
	Acclimation * Temperature * Relative humidity	168.5	8	21.1	9.01	< 0.001
	Error	1830	783	2.3		

4. Discussion

Organisms face increasingly divergent and overlapping abiotic stressors under climate change. However, physiological temperature tolerance is often singled as the most important driver to ectotherm fitness, population abundance and biogeography (Sinclair et al., 2012). Basal thermal resistance and plasticity under single or multiple stress factors across the entire ontogeny is important for maintaining fitness under heterogeneous environments (May et al., 2018). Our results show that the interaction across temperature, RH and developmental acclimation stage (ontogeny) significantly affected the thermal performance of adult *C. partellus* measured as CTLs, SCPs, CCRT and HKDT. This suggests that other factors such as humidity and life-stage may act in concert to influence overall insect thermal sensitivity under climate variability. Indeed, similar studies have shown that superior basal and plastic responses to environmental heterogeneity can drive thermal fitness traits in related invasive insect species upon introduction to novel environments (Nyamukondiwa and Terblanche, 2010; Gotcha et al., 2018; Mutamiswa et al., 2018a).

The current study showed a significant developmental stage acclimation effects on CTLs. Notably, larval acclimation at 23 °C; 85% RH recorded the lowest CT_{min} compared to pupal and adult acclimations under the same conditions. This indicates improved cold tolerance following larval acclimation relative to pupal and adult acclimations. In addition, this also shows that during larval acclimation there were improved physiological responses to thermal tolerance which were carried over to each stage of development till adult stage. Furthermore, it also suggests that physiological plastic responses acquired during development may give a survival and fitness advantage to *C. partellus* adults later in life when faced with stressful or changing environments (Chidawanyika and Terblanche, 2010). Our study also showed significant adult acclimation effects on CT_{max} with moths acclimated at 28 °C; 45% RH recording the highest CT_{max}. This upper thermal limit is higher than previously reported by Mutamiswa et al. (2018b) following adult acclimation at 28 °C; 65% RH. This finding indicates a higher level of plasticity following adult acclimation relative to pupal and larval acclimations, and following a lower RH (45%) relative to benign RH (65%) (see Mutamiswa et al., 2018b). This may point to a potential synergy between high temperature and low RH in improving heat tolerance. Indeed, this is not surprising since mechanisms associated with heat and desiccation tolerance may be overlapping, e.g. through improved expression of heat shock proteins (see Gibbs et al., 2003; Gotcha et al., 2018). As such, some overlap between the two stressors of high temperature and desiccation have been widely reported (e.g. Bubliy et al., 2012; Gotcha et al., 2018), associated with these likely overlapping protective mechanisms (cross tolerance) or signal pathways (cross talk) (Sinclair et al. (2013). Nevertheless, adult acclimation at 33 °C; 45% RH recorded the lowest CT_{max} indicating reduced heat tolerance. The reason for this decline is unknown but may be due to increased and irreversible cell damage associated with exposure to both stressful high temperature (33 °C) and low RH (45%) extremes, which may not allow insect recovery. This is however consistent with a

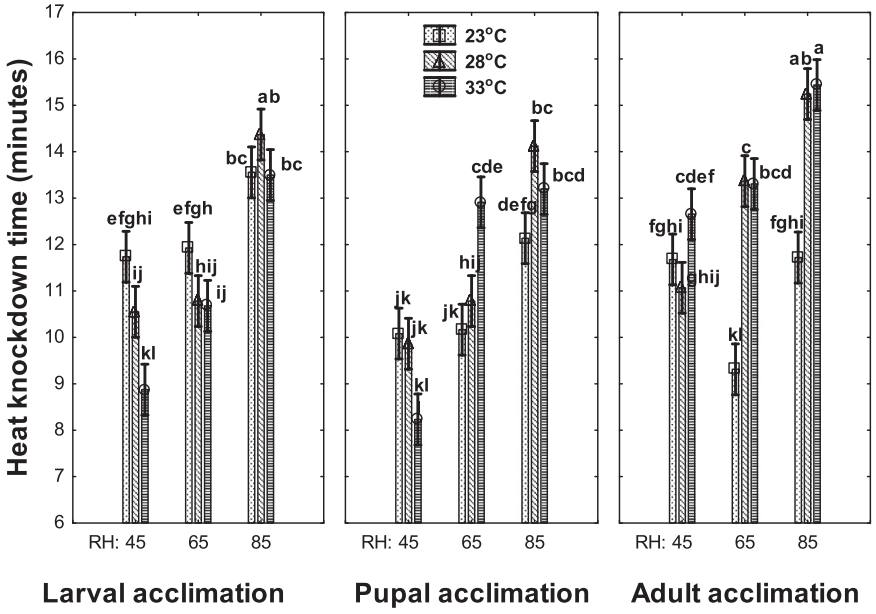

Fig. 5. Summary results of the effects of temperature (23, 28 and 33 °C), relative humidity (45%, 65% and 85%) and acclimation stage (larval, pupal and adult) on adult *C. partellus* heat knockdown time. Vertical bars represent 95% CLs (N = 30 per group). Means with the same letter are not statistically different.

previous study that reported impaired survival of tropical bed bug, *Cimex hemipterus* following adult acclimation at high temperatures and low humidity (33%) (How and Lee, 2014). Climate change is expected to increase ectotherm physiological stress through associated changes in abiotic environments (Andrew and Terblanche, 2013; Stathers et al., 2013; IPCC et al., 2014). Consistent with findings here, and for many ectotherms, temperature and precipitation interactions will physiologically limit organismal activity and fitness (Chown and Nicolson, 2004). And for invasive insects, variation in acclimation capacity across populations through genetic and ontogenic means may provide the necessary adaptive compensatory mechanisms for surviving the stress (Angilletta, 2009). Certainly, the variability in plasticity across developmental stages observed here may provide opportunities for selection that drives evolution of thermal stress tolerance under rapidly changing climates.

Although developmental and adult acclimations had significant effects on adult *C. partellus* CT_{min}, it had little effects on SCPs. Improved supercooling may be facilitated by rapid accumulation of cryoprotectants and extracellular agents such as polyols, sorbitol and sugars to maintain membrane function (Jones et al., 2008; Sinclair et al., 2015). Nevertheless, we found no significant differences in SCPs following larval, pupal and adult acclimations (Fig. 3). Factors affecting thermal acclimation are complex but increasingly becoming elucidated (Bar-Ziv and Scharf, 2018; Rohr et al., 2018). Specifically, acclimation detection may be trait and methodological context dependent. This may point to the notion that SCPs acclimatory magnitudes may not be easily detectable, at least in the context of the current study methodology. Indeed, behavioural traits e.g. locomotion or flight have been reported to respond more to acclimation than critical temperature endpoints (Krebs and Thompson, 2006), and as such, physiological ecologists have often used the former to unravel benefits of thermal acclimation (see Lachenicht et al., 2010). However, the weak acclimation responses to SCPs reported here corroborates findings that were reported on springtails (Slabber et al., 2007).

Chill coma recovery time represents the time required to regain consciousness following stressful low temperature, and a prolonged time in coma represents vulnerability under low stressful temperatures. Acclimation potential for CCRT means improvement in organism's environmental low temperature stress tolerance, thus minimizing opportunity costs likely associated with fitness benefits e.g. predator escape, foraging and mating (Sinervo et al., 2010). Similarly, inability to acclimate in synchrony with changing ambient conditions (e.g. low and high temperatures) has been linked directly to climate change related species and population collapse (Cohen et al., 2018). While previous studies showed no evidence of developmental acclimation effects on recovery time in *Drosophila melanogaster* (e.g. Colinet and Hoffman, 2012), our results report otherwise, with larval and pupal acclimations significantly improving CCRT. This result shows life-stage related differences in acclimation responses, in keeping with Rako and Hoffmann (2006), Maclean et al. (2017). The study by Colinet and Hoffmann et al. (2012) included temperature preconditioning as the only key treatment eliciting developmental acclimation. However, our study included both temperature and RH preconditioning. This therefore supports the notion that combined interactions of temperature and RH may have an additive effect on improving cold tolerance (CCRT) of adult *C. partellus*. Consistent with CT_{min} results, it appeared the interaction between temperature × RH also improved cold tolerance, measured as CCRT. For example, adult *C. partellus* that had been acclimated as larvae (23 °C; 85%RH) or adults (23 °C; 45%RH) recovered faster following chill coma, symbolising improved cold tolerance (CCRT) (see Fig. 4). This finding affirms the fitness benefits associated with interactive effects of acclimation temperature and RH, consistent with findings in *Acheta domesticus* (Lachenicht et al., 2010), *Melitaea cinxia* (Luo et al., 2014; Lei et al., 2016) and *Bombyx mori* (Xiao et al., 2017). While the interaction effects of high temperature (33 °C) and RH appeared to be negligible in larval acclimation, the benefits were more detectable under pupal and adult acclimation. For the latter, our results suggest the interactions of high temperature (33 °C) and high RH (65% and 85%) enhanced cold tolerance, suggesting synergistic interactive effects of the two stressors on temperature acclimation. This acclimation benefit was also more pronounced under adult than pupal acclimation (see Fig. 4), liking indication ontogenetic roles in the development of acclimatory responses.

Larval, pupal and adult acclimations at 28 °C; 85%RH generally improved heat tolerance (HKDT) relative to the other temperature × RH interactions; with tested organisms taking longer to be knocked down following exposure to acute high knockdown temperature (see Fig. 5). This indicates the fitness benefits associated with the interactive acclimation effects at high RH (85%) and temperature (28 °C) on adult *C. partellus* thermal fitness. Conversely, high temperature and low RH (33 °C; 45%RH) impaired heat tolerance (HKDT) following larval and pupal acclimations, pointing to thermal fitness costs (HKDT) associated with interactive potentially irreversible effects of high temperature and low RH stress. Previous studies reported that high temperatures and low RH have a detrimental effect on insect survival (Terblanche et al., 2007b, 2008). Given that the rate of water loss in insects is dependent on temperature and RH (Gibbs et al., 2003), our results are therefore in agreement with Bubliy et al. (2012), who reported that *D. melanogaster* exposed to drier environments were knocked down faster than those exposed to humid environments. Extreme temperatures and RH are expected under rapidly changing climates (Lobell et al., 2008; Stathers et al., 2013). As such, explaining interactive effects of changes in these abiotic environments and their effects on thermal fitness is important in explaining species persistence under climate change. It also appeared that acclimation at high temperature and RH (33 °C; 85% RH) generally improved heat tolerance (HKDT), i.e. took a prolonged time to be knocked down following acute heat stress, in keeping with previous findings (Gibbs et al., 2003; Bubliy et al., 2012). These differential responses may be due to variations in patterns of gene expression at different acclimation environments, which may upregulate molecular chaperone proteins that improve fitness at acute high temperatures (Feder and Hofmann, 1999).

While recent reports suggest the ecological relevance of experimental methodologies incorporating abiotic environmental fluctuations (Harris et al., 2018; Nyamukondiwa et al., 2018), we strongly feel the static experimental methodologies used here still provide accurate estimation of insect fitness responses under variable climates (Sinervo et al., 2017; Bar-Ziv and Scharf, 2018; Kellermann et al., 2018). Vapour pressure deficit (VPD), which is a measure of the drying power of the air is highly dependent on both temperature and RH (Bujan et al., 2016). While the current study did not incorporate VPD, it may likely have effects on the results reported here. Nevertheless, we conclude significant costs and benefits of interactive effects of climate stressors on thermal acclimatory ability in invasive *C. partellus*. While organismal acclimatory ability is adaptive and near ubiquitous (Gunderson et al., 2017), no general rule applies in predicting plasticity potential and breath thereof across different interacting factors. While theory suggest organisms from highly variable environments may have more acclimatory capacity (Angiletta, 2009; Renault et al., 2018), empirical evidence speaks otherwise (Angilletta, 2009). Nevertheless, current literature suggest body mass may drive thermal plasticity (Kingsolver and Huey 2008) and that there is a positive relationship between temperature plasticity and body size (Angilletta, 2009). While previous studies solely prioritized temperature in driving thermal acclimation, the current study showed interactive costs and benefits of developmental stage, temperature and RH in driving potential thermal plasticity. This therefore implies that, physiological ecologists should incorporate these factors and interactions thereof in predictive models looking at acclimatory capacities and how they may buffer ectotherms to temperature associated mortality in increasingly warming and variable abiotic environments. Adults that were developmentally and adult acclimated (across temperature × RH interactions) showed differential

thermal fitness levels indicating the critical roles of both temperature and RH in ectotherm survival under global climate change. This therefore warrants for the consideration of the role of ontogeny and multi-factors (including VPD) in better understanding of the impact of environmental stress on ectotherms, in particular on adaptive phenotypic plasticity.

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Declaration of interest

The authors declare they do not have any conflict of interests

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References

Andrew, N.R., Terblanche, J.S., 2013. The response of insects to climate change. In: Salinger, J. (Ed.), Living in a Warmer World: How a Changing Climate Will Affect Our Lives. David Bateman Ltd, Auckland, pp. 38–50.

Angilletta, M.J., 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis. Oxford University Press, New York, New York.

Arias, M.B., Poupin, M.J., Lardies, M.A., 2011. Plasticity of life-cycle, physiological thermal traits and Hsp70 gene expression in an insect along the ontogeny: effect of temperature variability. J. Therm. Biol. 36, 355–362.

Bar-Ziv, M.A., Scharf, I., 2018. Thermal acclimation is not induced by habitat-of-origin, maintenance temperature, or acute exposure to low or high temperatures in a pit-building wormlion (*Vermileo* sp.). J. Therm. Biol. 74, 181–186.

Bowler, K., Terblanche, J.S., 2008. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? Biol. Rev. 83, 339–355.

Bubliy, O.A., Kristensen, T.N., Kellermann, V., Loeschcke, V., 2012. Humidity affects genetic architecture of heat resistance in *Drosophila melanogaster*. J. Evolut. Biol. 25, 1180–1188. https://doi.org/10.1111/j.1420-9101.2012.02506.x.

Bujan, J., Yanoviak, S.P., Kaspari, M., 2016. Desiccation resistance in tropical insects: causes and mechanisms underlying variability in a Panama ant community. Ecol. Evol. https://doi.org/10.1002/ece3.2355.

Cavieres, G., Bogdanovich, J.M., Bozinovic, F., 2016. Ontogenetic thermal tolerance and performance of ectotherms at variable temperatures. J. Evolut. Biol. 29, 1462–1468.

Chidawanyika, F., Nyamukondiwa, C., Strathie, L., Fischer, K., 2017. Effects of thermal regimes, starvation and age on heat tolerance of the Parthenium Beetle *Zygogramma bicolorata* (Coleoptera: chrysomelidae) following dynamic and static protocols. PLoS One 12, e0169371. https://doi.org/10.1371/journal.pone.0169371.

Chidawanyika, F., Terblanche, J.S., 2011. Costs and benefits of thermal acclimation for codling moth, *Cydia pomonella* (Lepidoptera: tortricidae): implications for pest control and the sterile insect release programme. Evolut. Appl. 4, 534–544.

Cohen, J.M., Lajeunesse, M.J., Rohr, J.R., 2018. A global synthesis of animal phenological responses to climate change. Nat. Clim. Change 8, 224.

Colinet, H., Hoffman, A.A., 2012. Comparing phenotypic effects and molecular correlates of developmental, gradual and rapid cold acclimation responses in *Drosophila melanogaster*. Funct. Ecol. 26, 84–93.

Dixon, A.F., Honek, A., Kell, P., Kotela, M.A.A., Sizzling, A.L., Jarosik, V., 2009. Relationship between the minimum and maximum temperature thresholds for development of insects. Funct. Ecol. 23, 257–264.

Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response. Annu. Rev. Physiol. 61, 243–282.

Garland, T., Kelly, S.A., 2006. Phenotypic plasticity and experimental evolution. J. Exp. Biol. 209, 2344–2361.

Gerken, A.R., Eller, O.C., Hahn, D.A., Morgan, T.J., 2015. Constraints, independence, and evolution of thermal plasticity: probing genetic architecture of long-and short-term thermal acclimation. Proc. Natl. Acad. Sci. https://doi.org/10.1073/pnas.1503456112.

Getu, E.D., 2007. Comparative studies of the influence of relative humidity and temperature on the longevity and fecundity of the parasitoid, *Cotesia flavipes*. J. Insect Sci. 19, 1536–2442.

Gibbin, E.M., N'Siala, G.M., Chakravarti, L.J., Jarrold, M.D., Calosi, P., 2017. The evolution of phenotypic plasticity under global change. Sci. Rep. 7, 17253.

Gibbs, A.G., Fukuzato, F., Matzkin, L.M., 2003. Evolution of water conservation mechanisms in *Drosophila*. J. Exp. Biol. 206, 1183–1192. https://doi.org/10.1242/jeb.00233.

Gotcha, N., Terblanche, J.S., Nyamukondiwa, C., 2018. Plasticity and cross-tolerance to heterogeneous environments: divergent stress responses co-evolved in an African fruit fly. J. Evolut. Biol. 31, 98–110.

Gunderson, A.R., Dillon, M.E., Stillman, J.H., 2017. Estimating the benefits of plasticity in ectotherm heat tolerance under natural thermal variability. Funct. Ecol. 31, 529–1539.

Harris, R.M.B., Beaumont, L.J., Vance, T.R., Tozer, C.R., Remenyi, T.A., Perkins-Kirkpatrick, S.E., Mitchell, P.J., Nicotra, A.B., McGregor, S., Andrew, N.R., Letnic, M., Kearney, M.R., Wernberg, T., Hutley, L.B., Chambers, L.E., Fletcher, M.S., Keatley, M.R., Woodward, C.A., Williamson, G., Duke, N.C., Bowman, D.M.J.S., 2018. Biological responses to the press and pulse of climate trends and extreme events. Nat. Clim. Change 8, 579–587.

Hemmati, C., Moharramipour, S., Talebi, A.A., 2014. Effects of cold acclimation, cooling rate and heat stress on cold tolerance of the potato tuber moth *Phthorimaea operculella* (Lepidoptera: gelechiidae). Eur. J. Entomol. 111, 487–494.

Hoffmann, A.A., Chown, S.L., Clusella-Trullas, S., 2012. Upper thermal limits in terrestrial ectotherms: how constrained are they? Funct. Ecol. 27, 934–949.

Hoffmann, A.A., Parsons, P.A., 1991. Evolutionary Genetics and Environmental Stress. Oxford University Press, UK.

How, Y.F., Lee, C.Y., 2014. Effects of temperature and humidity on the survival and water loss of *Cimex hemipterus* (Hemiptera: Cimicidae). J. Med. Entomol. 47, 987–995.

IPCC, 2014. In: Core Writing Team, R.K., Pachauri, Meyer, L.A. (Eds.), Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland, pp. 151.

Jones, D.B., Giles, K.L., Elliot, N.C., 2008. Supercooling points of *Lysiphlebus testaceipes* and its host *Schizaphis graminum*. Environ. Entomol. 37, 1063–1068.

Jensen, D., Overgaard, J., Sørensen, J.G., 2007. The influence of developmental stage on cold shock resistance and ability to cold-harden in Drosophila melanogaster. J. Insect Physiol. 53, 179–186.

Kellermann, V., Hoffmann, A.A., Overgaard, J., Loeschcke, V., Sgrò, C.M., 2018. Plasticity for desiccation tolerance across *Drosophila* species is affected by phylogeny and climate in complex ways. Proc. R. Soc. Lond. B 285, 20180048.

Kelley, A.L., 2014. The role thermal physiology plays in species invasion. Conserv. Physiol. https://doi.org/10.1093/conphys/cou04527.

Khadioli, N., Tonnang, Z.E.H., Muchugu, E., Ong'amo, G., Achia, T., Kipchirchir, I., Kroschel, J., Le Ru, B., 2014a. Effect of temperature on the phenology of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), simulation and visualization of the potential future distribution of *C. partellus* in Africa under warmer temperatures through the development of life-table parameters. Bull. Èntomol. Res. 104, 809–822.

Khadioli, N., Tonnang, Z.E.H., Ong'amo, G., Achia, T., Kipchirchir, I., Kroschel, J., Le Ru, B., 2014b. Effect of temperature on the life history parameters of noctuid lepidopteran stemborers, *Busseola fusca* and *Sesamia calamistis*. Ann. Appl. Biol. https://doi.org/10.1111/aab.12157.

Klok, C.J., Chown, S.L., 2001. Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic kelp fly, Paractora druexi (Diptera: helcomyzidae). J. Insect Physiol. 47, 95–109.

Krebs, R.A., Thompson, K.A., 2006. Direct and correlated effects of selection on flight after exposure to thermal stress in *Drosophila melanogaster*. Genetica 128, 217–225.

Lachenicht, M.W., Clusella-Trullas, S., Boardman, L., Le Roux, C., Terblanche, J.S., 2010. Effects of acclimation temperature on thermal tolerance, locomotion performance and respiratory metabolism in *Acheta domestica* L. (Orthoptera: gryllidae). J. Insect Physiol. 56, 822–830.

Lagerspetz, K.Y., Vainio, L.A., 2006. Thermal behaviour of crustaceans. Biol. Rev. 81, 237–258.

Lei, Y., Wang, Y., Ahola, V., Luo, S., Xu, C., Wang, R., 2016. RNA sequencing reveals differential thermal regulation mechanisms between sexes of Glanville fritillary butterfly in the Tianshan Mountains, China. Mol. Biol. Rep. 43, 1423–1433.

Lobell, D.B., Burke, M.B., Tebaldi, C., Mastrandrea, M.D., Falcon, W.P., Naylor, R.I., 2008. Prioritising climate change adaptation needs for food security in 2030. Science 319, 607–610.

Lockwood, B.L., Gupta, T., Scavotto, R., 2018. Disparate patterns of thermal adaptation between life stages in temperate vs. tropical *Drosophila melanogaster*. J. Evolut. Biol. 31, 323–331.

Luo, S., Wong, S.C., Xu, C., Hanski, I., Wang, R., Lehtonen, R., 2014. Phenotypic plasticity in thermal tolerance in the Glanville fritillary butterfly. J. Therm. Biol. 42, 33–39.

Marais, E., Chown, S.L., 2008. Beneficial acclimation and the Bogert effect. Ecol. Lett. 11, 1027–1036.

Marais, E., Terblanche, J.S., Chown, S.L., 2009. Life-stage related differences in hardening and acclimation of thermal tolerance traits in the kelp fly, *Paractora dreuxi* (Diptera: helcomyzidae). J. Insect Physiol. 55, 36–343.

May, C., Hillerbrand, N., Thompson, L.M., Faske, T.M., Martinez, E., Parry, D., Agosta, S.J., Grayson, K.L., 2018. Geographic variation in larval metabolic rate between northern and southern populations of the invasive Gypsy moth. J. Insect Sci. 18, 1–7.

Milton, C.C., Partridge, L., 2008. Brief carbon dioxide exposure blocks heat hardening but not cold acclimation in *Drosophila melanogaster*. J. Insect Physiol. 54, 32–40.

Mutamiswa, R., Chidawanyika, F., Nyamukondiwa, C., 2017a. Dominance of spotted stemborer *Chilo partellus* Swinhoe (Lepidoptera: crambidae) over indigenous stemborer species in Africa's changing climates: ecological and thermal biology perspectives. Agric. For. Entomol. 115, 1–11. https://doi.org/10.1111/afe.12217.

Mutamiswa, R., Chidawanyika, F., Nyamukondiwa, C., 2017b. Comparative assessment of the thermal tolerance of spotted stemborer, *Chilo partellus* Swinhoe (Lepidoptera: crambidae) and its larval parasitoid, *Cotesia sesamiae* Cameron (Hymenoptera:

braconidae). Insect Sci. https://doi.org/10.1111/1744-7917.12466.
Mutamiswa, R., Chidawanyika, F., Nyamukondiwa, C., 2018a. Superior basal and plastic thermal responses to environmental heterogeneity in invasive exotic stemborer *Chilo partellus* Swinhoe over indigenous *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson. Physiol. Entomol. https://doi.org/10.1111/phen.12235.
Mutamiswa, R., Chidawanyika, F., Nyamukondiwa, C., 2018b. Thermal plasticity potentially mediates the interaction between host *Chilo partellus* Swinhoe (Lepidoptera: crambidae) and endoparasitoid *Cotesia flavipes* Cameron (Hymenoptera: braconidae) under rapidly changing environments. Pest Manag. Sci. 74. https://doi.org/10.1002/ps.4807.
Mwalusepo, S., Tonnang, H.E.Z., Massawe, E.S., Okuku, G.O., Khadioli, N., Johansson, T., Calatayud, P., Le Ru, B.P., 2015. Predicting the impact of temperature change on the future distribution of maize stemborers and their natural enemies along East African mountain gradients using phenology models. PLoS One 10, e0130427. https://doi.org/10.1371/journal.pone.0130427.
Nguyen, C., Bahar, M.H., Baker, G., Andrew, N.R., 2014. Thermal tolerance limits of diamondback moth in ramping and plunging assays. PLoS One 9, e87535. https://doi.org/10.1371/journal.pone.0087535.
Norhisham, A.R., Abod, F., Rita, M., Hakeem, K.R., 2013. Effect of humidity on egg hatchability and reproductive biology of the bamboo borer (*Dinoderus minutus* Fabricius). SpringerPlus 2, 9.
Nyamukondiwa, C., Chidawanyika, F., Machekano, H., Mutamiswa, R., Sands, B., Mgidiswa, N., Wall, R., 2018. Climate variability differentially impacts thermal fitness traits in three coprophagic beetle species. PLoS One 13 (6), e0198610.
Nyamukondiwa, C., Terblanche, J.S., 2010. Within-generation variation of critical thermal limits in adult Mediterranean and Natal fruit flies *Ceratitis capitata* and *Ceratitis rosa*: thermal history affects short-term responses to temperature. Physiol. Entomol. 35, 255–264.
Nyamukondiwa, C., Terblanche, J.S., 2009. Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): effects of age, gender and feeding status. J. Therm. Biol. 34, 406–414.
Nyamukondiwa, C., Weldon, C.W., Chown, S.L., le Roux, P.C., Terblanche, J.S., 2013. Thermal biology, population fluctuations and implications of temperature extremes for the management of two globally significant insect pests. J. Insect Physiol. 59, 1199–1211.
Ochieng, R.S., Onyango, F.O., Bungu, M.D.O., 1985. Improvement of techniques for mass culture of *Chilo partellus* (Swinhoe). Insect Sci. Appl. 6, 425–428.
Parmesan, C., 2006. Ecological and evolutionary responses to recent climate change. Annu. Rev. Ecol. Evol. Syst. 37, 637–669.
Raghu, S., Drew, R.A.I., Clarke, A.R., 2004. Influence of host plant structure and microclimate on the abundance and behaviour of a tephritid fly. J. Insect Behav. 17, 179–190.
Rako, L., Hoffmann, A.A., 2006. Complexity of the cold acclimation response in *Drosophila melanogaster*. J. Insect Physiol. 52 (1), 94–104.
Renault, D., Laparie, M., McCauley, S.J., Bonte, D., 2018. Environmental adaptations, ecological filtering, and dispersal central to insect invasions. Annu. Rev. Entomol. 63, 345–368.
Rohr, J.S., Civitello, D.J., Cohen, J.M., Roznic, E.A., Sinervo, B., Dell, A.I., 2018. The complex drivers of thermal acclimation and breath in ectotherms. Ecol. Lett. 21, 1425–1439.
Sinclair, B.J., Williams, C.M., Terblanche, J.S., 2012. Variation in thermal performance among insect populations. Physiol. Biochem. Zool. 594–606.
Sinclair, B.J., Ferguon, L.V., Salehipour-shirazi, G., MacMillan, H.A., 2013. Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. Integr. Comp. Biol. 545–556.
Sinclair, B.J., Coello Alvarado, L.E., Ferguson, L.V., 2015. An invitation to measure insect cold tolerance: methods, approaches, and workflow. J. Therm. Biol. 53, 180–197.
Sinervo, B., Mendez-De-La-Cruz, F., Miles, D.B., Heulin, B., Bastiaans, E., Villagrán-Santa Maclean, H.J., Kristensen, T.N., Overgaard, J., Sørensen, J.G., Bahrndorff, S., 2017. Acclimation responses to short-term temperature treatments during early life stages causes long lasting changes in spontaneous activity of adult *Drosophila melanogaster*. Physiol. Entomol. 42, 404–411.
Sithole, S.Z., 1989. Sorghum stemborers in South Africa. In International Workshop on sorghum stem borers, 17–19 November 1987, ICRISAT Patancheru, A.P., 502, 324, India.
Slabber, S., Worland, M.R., Leinaas, H.P., Chown, S.L., 2007. Acclimation effects on thermal tolerances of springtails from sub-Antarctic Marion Island: indigenous and invasive species. J. Insect Physiol. 53, 113–125.
Stathers, T., Lamboll, R., Mvumi, B.M., 2013. Postharvest agriculture in changing climates: its importance to African smallholder farmers. Food Secur. 5, 361–392.
Tamiru, A., Getu, E., Jembere, B., Bruce, T., 2012. Effect of temperature and relative humidity on the development and fecundity of *Chilo partellus* Swinhoe (Lepidoptera: crambidae). Bull. Èntomol. Res. 102, 9–15.
Tefera, T., Mugo, S., Tende, R., Likhayo, P., 2010. Mass Rearing of Stem borers, Maize weevil and Larger grain borer Insect Pests of Maize. CIMMYT. Nairobi.
Terblanche, J.S., Marais, E., Chown, S.L., 2007a. Stage-related variation in rapid cold hardening as a test of the environmental predictability hypothesis. J. Insect Physiol. 53, 455–462.
Terblanche, J.S., Deere, J.A., Clusella-Trullas, S., Janion, C., Chown, S.L., 2007b. Critical thermal limits depend on methodological context. In: Proceedings of the Royal Society of London B 274, pp. 2935–2942.
Terblanche, J.S., Clusella-Trullas, S., Deere, J.A., Chown, S.L., 2008. Thermal tolerance in a south-east African population of the tsetse fly *Glossina pallidipes* (Diptera, Glossinidae): implications for forecasting climate change impacts. J. Insect Physiol. 54, 114–127.
Vazquez, D.P., Gianoli, E., Morris, W.F., Bozinovic, F., 2015. Ecological and evolutionary impacts of changing climatic variability. Biol. Rev. 92, 22–42.
Vernon, P., Vannier, G., 1996. Developmental patterns of supercooling capacity in a subantarctic wingless fly. Experientia 52, 155–158.
Weldon, C.W., Terblanche, J.S., Chown, S.L., 2011. Time-course for attainment and reversal of acclimation to constant temperature in two *Ceratitis* species. J. Therm. Biol. 36, 479–485.
Xiao, W., Chen, P., Xiao, J., Wang, L., Liu, T., Wu, Y., Dong, F., Jiang, Y., Pan, M., Zhang, Y., Lu, C., 2017. Comparative transcriptome profiling of a thermal resistant vs. sensitive silkworm strain in response to high temperature under stressful humidity condition. PLoS One 12 (5), e0177641.
Yampolsky, L.Y., Zeng, E., Lopez, J., Williams, P.J., Dick, K.B., Colbourne, J.K., Pfrender, M.E., 2014. Functional genomics of acclimation and adaptation in response to thermal stress in *Daphnia*. BMC Genom. 15, 859.